

Application Serial No. 09/700,806
Amendment Under 37 C.F.R. § 1.116 dated November 1, 2005
Reply to Final Office Action of September 1, 2005

Amendments to the Claims:

This listing of claims will replace all prior versions and listings of claims in the application.

Listing of Claims:

1. (currently amended) A method of treating a nitric oxide (NO) associated disorder in a mammal, wherein the disorder is hypertension, thrombosis, angina, atherosclerosis, or heart failure, comprising administering to said mammal an effective amount of VEGF receptor agonist that exhibits selective binding affinity for a KDR receptor and induces NO production in the mammal, wherein the agonist comprises a VEGF variant having:

a) one or more amino acid substitutions at or between residues F17 to Y25, wherein at least one of M18, Y21, Q22, or Y25 is substituted; and

b) one or more amino acid substitutions at or between residues D63 to E67;
wherein the binding affinity of the agonist for FLT-1 receptor is reduced as compared to the binding affinity of native VEGF for FLT-1 receptor.

2-7. (canceled)

8. (original) The method of claim 1 wherein said mammal is a human.

9. (canceled)

10. (previously presented) The method of claim 1 wherein said effective amount of VEGF receptor agonist enhances nitric oxide production in said mammal.

11-13. (canceled)

14. (currently amended) A method of stimulating sustained production of endogenous NO in an endothelial cell, comprising exposing the endothelial cell to an effective amount of a VEGF receptor agonist that exhibits selective binding affinity for a KDR receptor and induces up-

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regulation of NO synthase (eNOS) in the endothelial cell, wherein the agonist comprises a VEGF variant having one or more amino acid substitutions in a loop containing FLT-1 contact residues D63, E64, and E67, wherein ~~at least one or more of~~ residues D63 and G65 ~~or~~ L66 are substituted and the binding affinity of the agonist for FLT-1 receptor is reduced as compared to the binding affinity of native VEGF for FLT-1 receptor.

15. (canceled)

16. (currently amended) The method of claim 14, wherein the VEGF variant comprises one or more amino acid substitutions at or between positions F17 to Y25 of the native VEGF sequence (SEQ ID NO: 4).

17. (withdrawn) The method of claim 16, wherein in VEGF variant comprises at least the following amino acid substitutions: M18E, Y21L, Q22R and Y25S.

18. (canceled)

19. (previously presented) The method of claim 14, wherein the amino acid substitution(s) comprises D63S, G65M, or L66R.

20-22. (canceled)

23. (previously presented) The method of claim 1, wherein the amino acid substitution(s) comprises D63S, G65M, or L66R.

24. (previously presented) The method of claim 23, wherein the amino acid substitutions comprise D63S, G65M, and L66R.

25. (previously presented) The method of claim 19, wherein the amino acid substitutions comprise D63S, G65M, and L66R.

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26-27. (canceled)

28. (currently amended) The method of claim ~~27~~1, wherein the amino acid substitution(s) comprises one or more of M18E, Y21L, Q22R, or Y25S.

29. (previously presented) The method of claim 28, wherein the amino acid substitutions comprise M18E, Y21L, Q22R, and Y25S.

30. (currently amended) The method of claim ~~27~~1, wherein the VEGF variant comprises one of the following combinations of amino acid substitutions:

- (a) M18E, D63S, G65M, and L66R;
- (b) Y21L, D63S, G65M, and L66R;
- (c) Q22R, D63S, G65M, and L66R;
- (d) Y25S, D63S, G65M, and L66R;
- (e) M18E, Y21L, D63S, G65M, and L66R;
- (f) M18E, Q22R, D63S, G65M, and L66R;
- (g) M18E, Y25S, D63S, G65M, and L66R;
- (h) Y21L, Q22R, D63S, G65M, and L66R;
- (i) Y21L, Y25S, D63S, G65M, and L66R;
- (j) Q22R, Y25S, D63S, G65M, and L66R;
- (k) M18E, Y21L, Q22R, D63S, G65M, and L66R;
- (l) M18E, Q22R, Y25S, D63S, G65M, and L66R;
- (m) Y21L, Q22R, Y25S, D63S, G65M, and L66R;
- (n) M18E, Y21L, Q22R, Y25S, and D63S;
- (o) M18E, Y21L, Q22R, Y25S, and G65M;
- (p) M18E, Y21L, Q22R, Y25S, and L66R;
- (q) M18E, Y21L, Q22R, Y25S, D63S, and G65M;
- (r) M18E, Y21L, Q22R, Y25S, D63S, and L66R;
- (s) M18E, Y21L, Q22R, Y25S, G65M, and L66R; or

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(t) M18E, Y21L, Q22R, Y25S, D63S, G65M, and L66R.

31. (withdrawn) The method of claim 16, wherein the VEGF variant comprises one of the following combinations of amino acid substitutions:

- (a) M18E, D63S, G65M, and L66R;
- (b) Y21L, D63S, G65M, and L66R;
- (c) Q22R, D63S, G65M, and L66R;
- (d) Y25S, D63S, G65M, and L66R;
- (e) M18E, Y21L, D63S, G65M, and L66R;
- (f) M18E, Q22R, D63S, G65M, and L66R;
- (g) M18E, Y25S, D63S, G65M, and L66R;
- (h) Y21L, Q22R, D63S, G65M, and L66R;
- (i) Y21L, Y25S, D63S, G65M, and L66R;
- (j) Q22R, Y25S, D63S, G65M, and L66R;
- (k) M18E, Y21L, Q22R, D63S, G65M, and L66R;
- (l) M18E, Q22R, Y25S, D63S, G65M, and L66R;
- (m) Y21L, Q22R, Y25S, D63S, G65M, and L66R;
- (n) M18E, Y21L, Q22R, Y25S, and D63S;
- (o) M18E, Y21L, Q22R, Y25S, and G65M;
- (p) M18E, Y21L, Q22R, Y25S, and L66R;
- (q) M18E, Y21L, Q22R, Y25S, D63S, and G65M;
- (r) M18E, Y21L, Q22R, Y25S, D63S, and L66R;
- (s) M18E, Y21L, Q22R, Y25S, G65M, and L66R; or
- (t) M18E, Y21L, Q22R, Y25S, D63S, G65M, and L66R.

32. (currently amended) A method of treating a nitric oxide (NO) associated disorder in a mammal, wherein the disorder is hypertension, thrombosis, angina, atherosclerosis, or heart failure, comprising administering to said mammal an effective amount of VEGF receptor agonist that exhibits selective binding affinity for a KDR receptor, wherein the agonist comprises a VEGF variant having two or more amino acid substitutions in a loop containing FLT-1 contact

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residues D63, E64, and E67, wherein at least one or more of residues D63 and G65 or L66 are substituted and the binding affinity of the agonist for FLT-1 receptor is reduced as compared to the binding affinity of native VEGF for FLT-1 receptor.

33. (previously presented) The method of claim 32, wherein the amino acid substitution comprises D63S, G65M, or L66R.

34. (previously presented) The method of claim 33, wherein the amino acid substitution comprises D63S, G65M, and L66R.

35. (previously presented) The method of claim 14, wherein upregulation of eNOS is sustained for more than 24 hours.

36. (previously presented) The method of claim 14, wherein upregulation of eNOS is sustained for at least 2 days.

37. (previously presented) The method of claim 14, wherein upregulation of eNOS is sustained for at least 3 days.

38. (previously presented) The method of claim 14, wherein upregulation of eNOS is sustained for at least 4 days.

39. (previously presented) The method of claim 1, wherein NO production is sustained for more than 24 hours.

40. (previously presented) The method of claim 1, wherein NO production is sustained for at least 2 days.

41. (previously presented) The method of claim 14, wherein NO production is sustained for at least 3 days.

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42. (previously presented) The method of claim 14, wherein upregulation of eNOS is sustained for at least 4 days.